2476-Pos Board B168
Excluded Volume of EF-Tu Greatly Reduces the Barrier to Accommodation for aa-tRNA in the Ribosome
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Functional rearrangements in biomolecular assemblies result from diffusion across an underlying energy landscape. During the process of accommodation in the ribosome, aminoacyl-tRNA (aa-tRNA) displaces the encoded amino acid ~90 Å from outside of the ribosome (A/T state) to the peptidyltransferase center (A/A state), where it is added into the nascent protein chain. This process takes place after an initial selection step consisting of GTP hydrolysis of ternary complex (aa-tRNA/EF-Tu/ GTP), which releases aa-tRNA from EF-Tu. High-resolution ribosomal crystal structures allow for atomistic models that accurately account for the configurational entropy of the aa-tRNA as it undergoes accommodation constrained by the excluded volume of both the accommodation corridor of the ribosome and EF-Tu. These structural models can be combined with approximate energy landscapes (structure-based models SBM) that encode the accommodated state as the global energy minimum. We have performed extensive characterization of the interplay between configurational entropy and effective enthalpy during the accommodation process through umbrella sampling of the aa-tRNA elbow displacement. These simulations highlight a structural role for EF-Tu in accommodation beyond initial selection. The excluded voume of EF-Tu reduces the stability of the A/T basin, lowering the barrier to aa-tRNA elbow accommodation by as much as 10kBT, depending on parameterization, and shifts the equilibrium towards to the A/A basin. This result, along with results showing that near-cognate aa-tRNA raises the accommodation barrier relative to cognate, suggests the possibility that the kinetic proofreading step is closely tied to EF-Tu dissociation.

2477-Pos Board B169
Translation in the RNA World: Insight from the Proto-Ribosome Model
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The fundamental role of RNA in the universal processes of transcription and translation leads to the hypothesis of an era where RNAs could serve as both genetic material and metabolic enzymes. According to the RNA world hypothesis, these early ribozymes might catalyze formation of small peptides. Evolutionary analysis of ribosome structures and sequences suggested fragments of a proto-ribosome, near the peptidyl-transferase center (PTC) might have functioned as such a ribozyme. Exploiting the structural and sequence symmetry of the PTC along with RNA structure-function relationships, computationally designed small protoribosome, with only 4% of the nucleotides in a modern bacterial ribosome, was proven to be stable throughout micro-second molecular dynamics simulations and incorporates freely diffusing adenosine-alanine substrates spontaneously into the peptidyl-tRNA binding site. Moreover, the model binds the charged tRNA mimics in both the aminoacyl- and peptidyl-tRNA binding sites and reorients the substrates into a transition intermediate favorable for peptide bond formation. The in silico designed proto-ribosome is put under experimental investigation using native polyacrylamide gel electrophoresis (native PAGE), which supports the assembly of the symmetric fragments. The results suggest a possible pathway for the ancient RNA world to make statistical proteins that could assist in the further buildup of a more complex translation apparatus.

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EF-G:A-Site Trna Distance Changes during Translocation
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Prokaryotic elongation factor G (EF-G) catalyzes the coupled translocations on the ribosome of mRNA and A- and P-site bound tRNAs during the polypeptide elongation cycle. However, the kinetic pathway of this important step has not been fully elucidated. Here we describe the application of single molecule fluorescence resonance energy transfer (smFRET) to probe the mechanism of this key process in real time. Attaching a fluorescent probe within domain IV of EF-G permits monitoring of FRET efficiencies to sites in both ribosomal protein L11 and A-site tRNA. Two different sites on L11, position 38 or position 87, as well as two different A-site tRNAs, Phe-tRNA^{Phe} or Val-tRNA^{Val} were labeled with Cy5. The EF-G:L11 FRET efficiency is constant during EF-G occupancy of the ribosome (300 - 400 ms), whereas the EF-G:tRNA FRET proceeds from high to intermediate to low FRET values. This indicates that EF-G:A-site tRNA distance increases progressively during translocation, with our results providing the first good estimates of the timing of such changes. For both tRNAs, the EF-G:tRNA pair spends most of its time in the intermediate FRET state, with shorter lifetimes in the high and low FRET states. Each transition in the FRET value corresponds to a relative distance change of about 5 to 7 Å, so in total, we capture a 12 Å relative movement of A-site tRNA away from EF-G during translocation. EF-G:A-site tRNA FRET is sensitive to buffer conditions, with high Mg^{2+} and polyamines stabilizing the high FRET state. In addition, the translocation inhibitors viomycin and spectinomycin block formation of the low FRET state, although spectinomycin allows more movement between EF-G and tRNA than viomycin. Supported by NIH grant GM080376 to YEG and BSC and AHA fellowship 12POST8910014 to CC.

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The Ribosomal Pre State in Stalled vs. Actively Translating Ribosomes
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During protein synthesis, tRNAs successively occupy three sites on the ribosome: A (aminoacyl), P (peptidyl), and E (exit). A proposed model based on observations of stalled ribosomes describes two distinct states before mRNA and tRNA translocation (PRE-translocation): a classical state in which the tRNAs occupy the same sites in the large and small ribosomal subunits (A/A and P/P), and a hybrid state in which the tRNAs occupy different sites in each subunit (A/P and P/E). To determine whether the exchange and partition between the classical and hybrid PRE states of stalled ribosomes is important for actively translating ribosomes, we conducted single molecule FRET experiments using ribosomes containing Cy5-labeled large subunit protein L11, and Val-tRNA^{Val}(Cy3) or Phe-tRNA^{Phe}(Cy3) in the A-site. We monitored FRET in stalled PRE complexes in the absence of EF-G•GTP, and during ongoing translation in the presence of EF-G•GTP. The mRNA contained a variable number (0, 4, or 8) of Tyr codons preceding Phe and Val codons. In stalled ribosomes, the histogram of L11-tRNA FRET values showed two peaks corresponding to the classical and hybrid states. In contrast, in actively translating ribosomes, the FRET histogram showed only one major peak, with a FRET efficiency that was intermediate between the two values for the stalled complexes. This distribution could correspond to an intermediate structure unique to active turnover, or to a transient state that, in the presence of EF-G, undergoes translocation more rapidly than it converts into the canonical classical and hybrid states. These results suggest that the PRE-translocation ribosomal state during active translation is distinct from the classical and hybrid states observed in stalled ribosomes, consistent with earlier results reported by our group (Rosenblum et al., JACS 2013). Supported by NIH grant GM080376 and AHA fellowship 12POST8910014 to CC.

2480-Pos Board B172
An mRNA Downstream Structure Promotes Frameshifting by Destabilizing the Hybrid State and Impeding the EF-G Driven Translocation Process
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Translational frameshifting occurs when a ribosome slips one or two nucleotides on a messenger RNA and generates a new sequence of amino acids. Many viral RNAs have programed frameshift-promoting signals to produce their proteins in the precise ratio needed for their viability. We used single-molecule fluorescence resonance energy transfer (smFRET) to study the dynamics of −1 programmed frameshifting by the *dnaX* gene in *E. coli*. The frameshifting mRNA has the usual three prokaryotic frameshifting signals: an internal Shine-Dalgarno sequence, a slippery sequence, and a stem loop. One round of translational elongation of the slippery sequence was characterized by the FRET changes between a Cy3-labeled L1 stalk in the 50S subunit and a Cy5-tRNA^{Lys} in the P-site. We observed that the downstream stem loop, a critical signal for efficient frameshifting, destabilizes the hybrid state and thus shifts the equilibrium toward to the classical state of pre-translocation complexes. Translocation catalyzed by EF-G was significantly slower in the frameshifting mRNA than in the non-frameshifting mRNA lacking the stem loop. Furthermore, pre-translocation complexes of the frameshifting mRNA underwent several transitions between the classical and hybrid states in the presence of EF-G prior to complete translocation, while the majority of the